PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 4 : C07G 11/00, A61K 35/00 // A61K 35/56

A2

(11) International Publication Number: WO 87/ 07610

(43) International Publication Date: 17 December 1987 (17.12.87)

(21) International Application Number: PCT/US87/01226

(22) International Filing Date: 1 June 1987 (01.06.87)

(31) Priority Application Numbers: 872,189
898,906
006,395

(32) Priority Dates: 9 June 1986 (09.06.86)
21 August 1986 (21.08.86)
23 January 1987 (23.01.87)

(33) Priority Country: US

(60) Parent Applications or Grants
(63) Related by Continuation
US 898,906 (CIP)
Filed on 21 August 1986 (21.08.86)
US 872,189 (CIP)
Filed on 9 June 1986 (09.06.86)
US 006,395 (CIP)
Filed on 23 January 1987 (23.01.87)

(71) Applicant *(for all designated States except US)*: UNIVERSITY OF ILLINOIS [US/US]; 506 South Wright Street, Urbana, IL 61801 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : RINEHART, Kenneth, L. [US/US]; 1306 South Carle Avenue, Urbana, IL 61801 (US). HOLT, Tom, G. [US/US]; 450 Paddock Drive West, Savoy, IL 61874 (US).

(74) Agent: WELCH, Lawrence, T.; Patent Law Department, The Upjohn Company, Kalamazoo, MI 49001 (US).

(81) Designated States: AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent), DK, FI, FR (European patent), GB (European patent), IT (European patent), JP, KR, LU (European patent), NL (European patent), NO, SE (European patent), US, US, US.

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: ECTEINASCIDINS 729, 743, 745, 759A, 759B AND 770

(57) Abstract

Novel compounds ecteinascidins 729, 743, 745, 759A, 759B, and 770 having antibacterial and antitumor properties.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria
AU	Australia
BB	Barbados
BE	Belgium
BG	Bulgaria
BJ	Benin
BR	Brazil
CF	Central African Republic
CG	Congo
CH	Switzerland
CM	Cameroon
DE	Germany, Federal Republic of
DK	Denmark
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
HU	Hungary
IT	Italy
JP	Japan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
LI	Liechtenstein
LK	Sri Lanka
LU	Luxembourg
MC	Monaco
MG	Madagascar
ML	Mali
MR	Mauritania
MW	Malawi
NL	Netherlands
NO	Norway
RO	Romania
SD	Sudan
SE	Sweden
SN	Senegal
SU	Soviet Union
TD	Chad
TG	Togo
US	United States of America

ECTEINASCIDINS 729, 743, 745, 759A, 759B AND 770

DESCRIPTION

The present application relates to novel compositions of matter. More particularly, the present application relates to novel antibacterial agents designated as ecteinascidin 729, 743, 745, 759A, 759B, and 770. These compounds are extracted from the marine tunicate Ecteinascidia turbinata, which is a well-known and readily available tropical marine invertebrate. Biological activity has been assigned previously to extracts of E. turbinata; see, for example, M. M. Sigal et al., "Anticellular and Antitumor Activity of Extracts from Tropical Marine and Vertebrates," in Food-Drugs from Sea Proceedings (1969), Youngken, H.W., Jr., Ed., Marine Technology Society,: Washington, D.C., 1970, pp 281-294; Lichter, W. et al., "Biological Activities Exerted by Extracts of Ecteinascidia turbinata," in Food-Drugs from the Sea Proceedings (1972), Worthen, L.R., Ed., Marine Technology Society: Washington, D.C., 1973, pp 117-127; Lichter, W., et al., "Inhibition of DNA Synthesis by Ecteinascidia turbinata Extracts (ETE)", in Food-Drugs from the Sea Proceedings, 1974, Webber, H.H., Ruggieri, G.D., Eds., Marine Technology Society: Washington, D.C., 1976, pp. 395-401; and Lichter, W. et al., "Immunomodulation by Extracts of Ecteinascidia turbinata", in Drugs and Food From the Sea, Kaul, P.N., Sindermann, C.J., Eds., The University of Oklahoma: Norman, OK, 1978, pp. 137-144.

INFORMATION DISCLOSURE

Extracts from Ecteinascidia turbinata are known, as described above. Certain of these extracts are known to have biological activity.

SUMMARY OF THE INVENTION

The present invention particularly provides:

(1) Ecteinascidin 729, having the following characteristics: R_f = 0.28 (TLC, 5.02, 3:1 ethyl acetate-methanol), 0.26 (9:1 chloroform-methanol); HPLC retention time, 15.7 min (Whatman Partisil 10 ODS-3, 10 x 250 mm, 70:30 methanol-aqueous Tris (0.05 M), 2.8 mL/min); UV max (CH_3OH), 202 nm (ε 61 000), 244 (sh) (11 000), 283 (5 000), 289 (4 700), (0.1 N HCl) 204 (61 000), 244 (sh) (9 600), 283 (4 800), 289 (4 500), (0.1 N KOH) 215 (33 800), 258 (8 200), 290 (6 400); IR (CCl_4) 3555, 3535, 2953, 2927, 2855, 1770, 1742, 1504, 1466, 1462, 1454, 1432, 1369, 1239, 1196, 1168, 1122, 1100, 1086, 1054, 1032, 997, 960 cm^{-1}; ^1H NMR (360 MHzCDCl$_3$) δ 6.63 (s, 1H), 6.48 (s,

1H), 6.44 (s, 1H), 6.04 (d, J = 0.7 Hz, 1H), 5.95 (d, J = 0.9 Hz, 1H), 5.15 (d, J = 10.7 Hz, 1H), 4.84 (bs, 1H), 4.52 (bs, 1H), 4.48 (bs, 1H), 4.38 (d, J = 4.9 Hz, 1H), 4.04 (d, J = 11 Hz, 1H), 3.78 (s, 3H), 3.62 (s, 3H), 3.61 (m, 2H), 3.10 (m, 1H), 3.02 (bs, 1H), 2.90 (m, 1H), 2.80 (m, 1H), 2.60 (m, 1H), 2.50 (m, 1H), 2.35 (m, 1H), 2.31 (s, 3H), 2.27 (s, 3H), 2.20 (m, 2H), 2.03 (s, 3H); FABMS, m/z (rel intensity) 730(30), 495(2), 493(2), 481(2), 479(2), 463(4), 461(2), 449(4), 205(8), 204(8), 190(8); FABMS m/z 730.2493; B/E linked scan on m/z 729, m/z 711, 696, 683, 509, 495, 481, 479, 461, 449; optical relation $[\alpha]_D^{25}$ + 112° (c 0.01, CH_3OH);

(2) Ecteinascidin 743, having the following characteristics: Beige solid, R_f = 0.58 (3:1 ethyl acetate-methanol), 0.44 (9:1 chloroform-methanol); HPLC retention time, 18.8 minutes (Whatman Partisil 10 ODS-3, 10 x 250 mm, 70:30 methanol-aqueous Tris (0.05 M), 2.8 mL/min); UV max (CH_3OH) 202 nm (ε81 000), 240 (sh) (15 000), 284 (6 600), 289 (6 400), (0.1 N HCl) 205 (76 000), 240 (sh) (12 000), 285 (7 500), 289 (7 200), (0.1 N KOH) 216 (50 000), 256 (12 700), 290 (9 000). IR max (CCl_4) 3549, 3530, 2992 (weak), 2929, 2848, 2803 (weak), 1764, 1739, 1597 (weak), 1511, 1501, 1460, 1445, 1425, 1365, 1350, 1195, 1160, 1115, 1102, 1098, 1082, 1058, 1048, 1024, 990, 950, 915, 907 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 6.62 (s, 1H), 6.48 (s, 1H), 6.46 (s, 1H), 6.03 (d, J = 1.2 Hz, 1H), 5.95 (d, J = 1.3 Hz, 1H), 5.71 (bs, exchanges, 1H), 5.14 (dd, J = 0.9, 11.3 Hz, 1H), 4.83 (bs, 1H), 4.50 (d, J = 3.3 Hz, 1H), 4.18 (d, J = 4.2 Hz, 1H), 4.06 (dd, J = 2.5, 11.3 Hz, 1H), 3.81 (s, 3H), 3.63 (s, 3H), 3.59 (bd, J = 4.4 Hz, 1H), 3.23 (bd, J = 6.5 Hz, 1H), 3.14 (ddd, J = 11, 10, 4 Hz, 1H), 2.91 (bd, J = 18 Hz, 1H), 2.88 (dd, J = 9, 18 Hz, 1H), 2.82 (m, 1H), 2.62 (ddd, J = 16, 10, 4 Hz, 1H), 2.49 (ddd, J = 16, 4, 4 Hz, 1H), 2.37 (bd, J = 13.9 Hz, 1H), 2.33 (s, 3H), 2.28 (s, 3H), 2.19 (s, 3H), 2.18 (d, J = 13.9 Hz, 1H), 2.04 (s, 3H); ^{13}C NMR (75.4 MHz and 125.7 MHz, $CDCl_3$) δ 9.6 (q), 15.7 (q), 20.4 (q), 24.0 (q), 28.7 (t), 39.6 (t), 41.3 (q), 42.1 (t), 42.1 (d), 54.8 (q), 55.0 (d), 55.9 (d), 57.7 (d), 57.8 (d), 60.2 (q), 61.3 (t), 64.6(s), 82.0 (d), 101.6 (t), 109.8 (d), 112.5, 114.1 (d), 115.9, 118.1(s), 120.9 (d), 121.9(s), 126.0(s), 129.2(s), 129.2(s), 131.5(s), 140.5(s), 141.3(s), 143.0(s), 144.3(s), 144.5(s), 145.1(s), 147.7(s), 168.3(s), 172.5(s); FABMS m/z (rel intensity) 744.2648 (100), 699.2766 (4), 613 (10), 495.2064 (15), 477.1978 (15), 475 (9), 463.1837 (25), 218(39), 204.1027 (71).

LC/FABMS m/z (rel intensity) 744 (34), 495 (12), 493 (16), 477 (14), 475 (10), 463 (14), 234 (42), 218 (64), 204 (100), 189 (62), 174 (28), 160 (22); EIMS m/z 217.0737305, 191.0941620, 176.0696716. ESCA (mole percent) C(73.1), O(20.4), N(5.2), S(1.3), optical rotation $[\alpha]_D^{25}$ + 1140 (c 0.1, CH_3OH);

(3) Ecteinascidin 745, having the following characteristics: R_f = 0.42 (3:1 ethyl acetate-methanol, 0.38 (9:1 chloroform-methanol). HPLC retention time, 29.8 min (Whatman Partisil 10 ODS-3, 10 x 250 mm, 70:30 methanol-aqueous Tris (0.05 M), 2.8 mL/min). UV max (CH_3OH) 202(ε52 000), 240(sh, 11 000), 281 (5 600), 287 (5 400), (0.1 N HCl), 204(51 000), 240 (sh, 9 500), 281 (5 200), 287 (5 200), (0.1 N KOH), 215 (36 000), 254 (8 500), 290 (5 900), 298 (5 800). IR (CCl_4 3554, 3535, 2955, 2927, 2871, 2855, 1770, 1744, 1518, 1507, 1270, 1238, 1195, 1163, 1088, 1056 cm^{-1}. ^1H NMR (360 MHz, $CDCl_3$) δ 6.62 (s, 1H), 6.52 (s, 1H), 6.47 (s, 1H), 6.02 (d, J = 1.2 Hz, 1H), 5.97 (d, J = 1,2 Hz, 1H), 5.74 (bs, exchanges, 1H), 5.14 (d, J = 11.2 Hz, 1H), 4.50 (bs, 1H), 4.29 (bt, J = 7 Hz, 1H), 4.22 (bs, 1H), 4.11 (dd, J = 11, 2 Hz, 1H), 3.80 (s, 3H), 3.68 (s, 1H), 3.63 (s, 3H), 3.31 (dd, J = 11, 2 1H), 3.23 (bs, 1H), 3.11 (m, 2H), 2.93 (m, 2H), 2.69 (m, 2H), 2.54 (m, 2H), 2.44 (d, J = 17 Hz, 1H), 2.33 (s, 3H), 2.28 (s, 3H), 2.18 (s, 3H), 2.13 (m, 1H), 2.04 (s, 3H); FABMS m/z (rel intensity) 746.2775 (100), 699 (8), 631 (8), 269 (8), 495 (19), 479 (42), 477 (52), 463 (36), 205 (64), 204 (64); LC/FABMS m/z (rel intensity) 746 (44), 495 (18), 477 (20), 463 (32), 218 (42), 204 (100), 189 (62), 176 (32), 160 (20), optical rotation $[\alpha]_D^{25}$ + 50° (c 0.1, CH_3OH); UV max (CH_3OH) 202(ε52 000), 240(sh, 11 000), 281 (5 600), 287 (5 400), (0.1 N HCl), 204(51 000), 240 (sh, 9 500), 281 (5 200), 287 (5 200), (0.1 N KOH), 215 (36 000), 254 (8 500), 290 (5 900), 298 (5 800);

(4) Ecteinascidin 759A, having the following characteristics: LC/FABMS m/z (rel intensity) 760 (26), 509 (12), 493 (12), 463 (24), 449 (16), 246 (26), 232 (32), 224 (62), 218 (52), 204 (100), 189 (56), 174 (18), 160 (16). R_f = 0.6 (3:1 ethyl acetate-methanol), 0.3 (9:1 chloroform-methanol); HPLC retention time, 11.0 min (Whatman Partisil 10 ODS-3, 10 x 250 mm, 70:30 methanol-aqueous tris (0.05 M), 2.8 mL.min); UV max (CH_3OH) 203 nm (ε x 43 000 43), 250 (sh) (6 500), 281 (3 000), 288 (2 600), (0.1 N HCl) 205 (44 000), 250 (sh) (7 600), 281 (4 500), 288 (4 400), (0.1 N KOH) 216 (39 000), 249 (9 300), 294

(4 600); IR (CCl_4) 3696, 3555, 3532, 2926, 2854, 1770, 1744, 1670, 1466, 1252, 1240, 1194, 1091 cm^{-1}; FABMS m/z (rel intensity) 760.2563 (58), 581 (25), 493 (16), 463 (80), 461 (100) optical rotation $[\alpha]_D^{25}$ + 130° (c 0.05, CH_3OH);

(5) Ecteinascidin 759B, having the following characteristics: LC/FABMS m/z (rel intensity) 760 (38), 508 (8), 493 (18), 463 (26), 475 (14), 248 (30), 234 (48), 218 (86), 204 (100), 189 (56), 176 (26), 160 (32). R_f = 0.6 (3:1 ethyl acetate-methanol), 0.3 (9:1 chloroform-methanol); HPLC retention time, 13.9 min (Whatman Partisil 10 ODS-3, 10 x 250 mm, 70:30 methanol-aqueous tris (0.05 M), 2.8 mL/min); UV max (CH_3OH) 208 nm (ε x 60), 288 (4 800), 293 (4 500), (0.1 N HCl) 209 (64 000), 288 (7 100), 293 (7 100), (0.1 N KOH) 220 (45 000), 260 (10 000), 298 (7 600); IR (CCl_4) 3555, 2933, 1770, 1743, 1590, 1514, 1465, 1453, 1446, 1431, 1368, 1356, 1330, 1288, 1264, 1240, 1193, 1163, 1124, 1110, 1089, 1032, 1006, 821 cm^{-1}; FABMS m/z (rel intensity) 760.2519 (100), 744 (71), 730 (19), 493 (29), 477 (43), 463 (76); optical rotation $[\alpha]_D^{25}$ + 167° (c 0.1, CH_3OH); and

(6) Ecteinascidin 770, having the following charcteristics: R_f = 0.6 (3:1 ethyl acetate-methanol), 0.3 (9:1 chloroform-methanol); HPLC retention time, 12.0 (Whatman Partisil 10 ODS-3, 10 x 250 mm, 70:30 methanol-aqueous tris (0.05 M), 2.8 mL/min); UV max (CH_3OH) 342 nm (ε 3 200), 329 (3 900, 299 (22 000), 263 (25 000), 240 (58 000), 234 (55 000), 216 (66 000), (0.1 N HCl) 342 (4 900), 329 (5 700), 299 (24 000), 263 (29 000), 240 (58 000), 234 (57 000), 216 (71 000), (0.1 N KOH) 342 (3 700), 329 (4 900), 299 (22 000), 263 (28 000), 240 (58 000), 234 (57 000), 227 (57 000); IR (CCl_4) 3555, 3535, 3484, 2929, 2910, 1770, 1742, 1607, 1516, 1509, 1504, 1494, 1462, 1450, 1433, 1325, 1237, 1193 cm^{-1}; FABMS, m/z (rel intensity) 771.2682(48), 760(8), 744(20), 723(12), 613(14), 488(12), 463(14), 461(20), 205(50), 204(80).

The organism from which the ecteinascidins were extracted is a marine colonial tunicate identified as Ecteinascidia turbinata by Dr. Francoise Lafargue, Universite de Paris VI, Laboratoire Arago, Banyuls-sur-Mer, France. E. turbinata belongs to the family Perophoridae, suborder Phlebobranchia, order Enterogona, class Ascidiacea, subphylum Tunicata, phylum Chordata. Detailed descriptions of this readily available organism can be found in the following references:

1. W.G. VanName, "The Ascidians of the Bermuda Islands," Trans. Conn. Acad. Arts Sci., 11, 325-412 (1902). See pages 338-339 for a description of E. turbinata.

2. W.G. VanName, "The North and South American Ascidians," Bull. Amer. Museum Nat. Hist., 84, 1-476 (1945). See plate 20, text figures 82A, 85, 86, and pages 169-171 for a complete description of E. turbinata and a comprehensive list of previous references.

3. H.H. Plough, "Sea Squirts of the Atlantic Continental Shelf from Maine to Texas," Johns Hopkins University Press, 1978, Baltimore, MD. See text figures 13, 30e, and pages 21-22, 54, and 68 for descriptions of E. turbinata.

E. turbinata is common and widely distributed in the Caribbean. It is conspicuous on account of the large size and often bright orange color of the colonies. A colony consists of a dense cluster of elongated, club-shaped zooids, which are connected at their tapered bases by a network of stolons that adheres to the surface of the object on which the colony grows. Colonies are found in shallow water (0-20 feet) growing on mangrove roots, sponges, rocks, shells, turtle grass, bridge pilings, etc., or on bottom sand or stone. The animals are easily collected by wading, snorkeling, or SCUBA techniques.

Samples have been obtained in the following locations:

1. On islands and shores of the Indian River near the Smithsonian Tropical Research Center, Harbor Branch Foundation, Fort Pierce, Florida, 27° 27′ N by 80° 20′ W.

2. Between No Name Key and Big Pine Key (especially on wooden bridge pilings), Florida, 24° 42′ N by 81° 21′ W.

3. In the Sunshine Key Resort boat harbor of Ohio Key, Florida, 24° 40′ N by 81° 14′ W.

4. In the keys near St. George's Caye and Drowned Caye, Belize, 17.5° N by 88° W.

The compounds of this invention, extracted from E. turbinata, exhibit antibacterial properties and thus are useful alone or in combination with other antibacterial agents to prevent the growth of or reduce the number of susceptible bacteria in many environments.

Certain compounds of the present invention have been shown to inhibit L1210 cells and P388 leukemia cells. Thus, the compounds of this invention are useful as antitumor agents. Therefore, they are

useful to inhibit the growth of tumor cells in mammals exhibiting such tumor cells. Illustratively, dosage levels of the administered active ingredients can be intravenous, 0.1 to about 200 mg/kg; intraperitoneal, 1 to about 500 mg/kg; subcutaneous, 1 to about 500 mg/kg; intramuscular, 1 to about 500 mg/kg; oral, 5 to about 1000 mg/kg; intranasal instillation, 5 to about 1000 mg/kg; and aerosol, 5 to about 1000 mg/kg of animal (body) weight.

The compounds of the present invention are preferably presented for administration to humans and animals in unit dosage forms, such as tablets, capsules, pills, powder, granules, suppositories, syrup, sterile parenteral solutions with suspensions, sterile non-parenteral solutions with suspensions, and oral solutions or suspensions and the like containing high active quantities of the active ingredient. Those of ordinary skill in the art of pharmaceutical compositions can readily formulate compounds of the present invention into appropriate pharmaceutical compositions.

The administration of the compounds claimed herein is useful to inhibit the growth of cancer cells in animals or humans bearing a neoplastic disease, for example, acute myelocystic leukemia, acute lymphocystic leukemia, malignant melanoma, adenocarcinoma of the lung, neuroblastoma, small cell carcinoma of the lung, breast carcinoma, colon carcinoma, bladder carcinoma, and the like.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present is an invention as seen more fully by the examples given below.

Example 1 (Isolation and Purification)

The isolation and purification procedure was monitored at each step by performing thin-layer chromatography (TLC), in vitro antimicrobial assays, in vitro cytotoxicity assays, and bioautography on in vitro microbial organisms and tissue cell lines.

Countercurrent chromatography (CCC) employed the Ito Multi-Layer Coil Separator-Extractor with a #10 coil, a Milton Roy pump, and a LKB Uvicord II Ultraviolet (UV) detector (254, 280 nm; 3-mm flow cell). Medium pressure liquid chromatography (MPLC) was carried out with a Milton Roy pump, Ace Glass Michel-Miller Chromatography Columns, and a LKB Uvicord II UV detector (280 nm, 3-mm flow cell). Analytical TLC and bioautography were carried out on E. Merck silica gel 60 F-254 analytical TLC plates. The plates were developed in 3:1

ethyl acetate-methanol or 9:1 chloroform-methanol solvent systems and visualized with iodine vapor or a 5% solution of phosphomolybdic acid in ethanol. UV spectra were obtained in methanol on a Perkin-Elmer Lambda 3 spectrophotometer. Infrared (IR) spectra were obtained in carbon tetrachloride or chloroform on a Nicolet 7000 Fourier Transform (FT)-IR. High performance liquid chromatography (HPLC) was carried out on a Whatman Partisil 10 ODS-3 column (10 x 250 mm) and employed a Beckman 110A solvent pump and a Beckman 153 UV detector (254 nm).

Nuclear magnetic resonance (NMR) spectra were obtained on Bruker 500, General Electric 300, and Nicolet NT-360 spectrometers. Chemical shifts for ^{1}H and ^{13}C NMR spectra are reported in ppm from tetramethylsilane. Low- and high-resolution fast atom bombardment (FAB) mass spectra were obtained on VG ZAB and VG Micromass 7070 mass spectrometers. Low- and high-resolution electron ionization (EI) mass spectra were carried out on a VG ZAB mass spectrometer. Liquid chromatography/FAB mass spectrometry (LC/FABMS) employed an Alltech C_{18} microbore HPLC column (10 μm, 1 x 250 nm) with a Beckman 114 solvent pump, a modified VG moving belt interface for operation in the FAB mode, and a VG ZAB mass spectrometer.

Ecteinascidia turbinata was collected in the Florida Keys at a depth of 0.2 to 15 feet. The sample was frozen until it was extracted by the following procedure. (Refer to Chart A.)

A sample of frozen tunicate (30.5 kg, wet weight) was thawed at room temperature and separated by coarse filtration into liquid and solid (6.3 kg) portions. A sample of the solid portion (3.8 kg) was extracted 12 times with methanol in a blender. The residue was filtered after each extraction to give a total of 20 L of methanol extract. The methanol extract was partitioned into an upper "organic" layer and a lower "polar" layer by adding 1 N aqueous $NaNO_3$ (6 L) and toluene (4 L). The aqueous phase was extracted an additional 3 times with toluene (4 L), and the toluene extracts were combined and evaporated under reduced pressure to give 26.9 g of a dark green oil. The resulting aqueous phase was extracted with dichloromethane (9 x 2L), and these extracts were combined and evaporated under reduced pressure to yield 15.9 g of a dark green solid.

The dichloromethane extract was solubilized successively with

dichloromethane and methanol, leaving a 5.3-g insoluble beige portion. The soluble portion was evaporated under reduced pressure to give a 10.6 g dark green solid. This material was triturated successively with hexane (20 x 20 mL) and dichloromethane (20 x 20 mL). The dichloromethane triturate (1.02 g) was purified by CCC (2 x 0.51 g) using a cyclohexane-chloroform-methanol-water solvent system (1:2:4:2) to give 8 fractions. Fractions were evaluated by TLC bioautography.

An early bioactive CCC fraction (300 mg) was chromatographed on a CHP-20 porous polymer MPLC column (18 x 350 mm) using a methanol-aqueous tris(hydroxymethyl)aminomethane (tris) (0.05 M) step gradient (85:15, 90:10, 95:5, 100:0). The chromatography was followed by UV detection (280 nm). Fractions were collected and tris was removed using a C_{18} Sep-Pak, eluting first with water (to remove tris), then with methanol (to recover sample). The major bioactive component was purified further by HPLC (Whatman Partisil 10 ODS-3, 10 x 250 mm) using a 70:30 methanol-aqueous tris (0.05 M) solvent system to yield, after tris removal, ecteinascidin 743 (27 mg) and ecteinascidin 745 (4.3 mg).

A later bioactive CCC fraction (69 mg) was chromatographed on a CHP-20 porous polymer MPLC column (18 x 350 mm) using a methanol-aqueous tris (0.05 M) step gradient (80:20, 85:15, 90:10, 100:0). The chromatography was followed by UV detection (280 nm). Tris was removed from the fractions as above. The major bioactive component was purified further by HPLC (Whatman Partisil 10 ODS-3, 10 x 250 mm) using a 70:30 methanol-aqueous tris (0.05 M) solvent system to yield, after tris removal, ecteinascidin 729 (2.5 mg).

Example 2

E. turbinata was extracted in a procedure similar to Example 1. A sample of the dichloromethane triturate (2.94 g) of the dichloromethane extract was purified by CCC (6 x 0.49 g) using a cyclohexane-chloroform-methanol-water solvent system (1:2:4:2) to give 10 fractions. Fractions were evaluated by TLC bioautography.

An early bioactive CCC fraction (200 mg) was further purified by CCC using a hexane-ethyl acetate-methanol-water (1:1:1:1) solvent system. A bioactive fraction (30 mg) was chromatographed on a CHP-20 porous polymer MPLC column (18 x 350 mm) using a methanol-water solvent system (85:15) buffered to pH 8.8 with 0.1% triethylamine-

acetic acid. The chromatography was followed by UV detection (280 nm). The major bioactive peak was collected (15.3 mg) and analyzed by LC/FABMS. LC/FABMS indicated one major component, ecteinascidin 743, and 3 minor components, ecteinascidin 745, ecteinascidin 759A, and ecteinascidin 759B.

Physical Characterization of the Ecteinascidins

Ecteinascidin 729 had: R_f = 0.28 (TLC, 5.02, 3:1 ethyl acetate-methanol), 0.26 (9:1 chloroform-methanol); HPLC retention time, 15.7 min (Whatman Partisil 10 ODS-3, 10 x 250 mm, 70:30 methanol-aqueous Tris (0.05 M), 2.8 mL/min); UV max (CH_3OH), 202 nm (ϵ 61 000), 244 (sh) (11 000), 283 (5 000), 289 (4 700), (0.1 N HCl) 204 (61 000), 244 (sh) (9 600), 283 (4 800), 289 (4 500), (0.1 N KOH) 215 (33 800), 258 (8 200), 290 (6 400); IR (CCl_4) 3555, 3535, 2953, 2927, 2855, 1770, 1742, 1504, 1466, 1462, 1454, 1432, 1369, 1239, 1196, 1168, 1122, 1100, 1086, 1054, 1032, 997, 960 cm^{-1}; ^1H NMR (360 MHz$CDCl_3$) δ 6.63 (s, 1H), 6.48 (s, 1H), 6.44 (s, 1H), 6.04 (d, J = 0.7 Hz, 1H), 5.95 (d, J = 0.9 Hz, 1H), 5.15 (d, J = 10.7 Hz, 1H), 4.84 (bs, 1H), 4.52 (bs, 1H), 4.48 (bs, 1H), 4.38 (d, J = 4.9 Hz, 1H), 4.04 (d, J = 11 Hz, 1H), 3.78 (s, 3H), 3.62 (s, 3H), 3.61 (m, 2H), 3.10 (m, 1H), 3.02 (bs, 1H), 2.90 (m, 1H), 2.80 (m, 1H), 2.60 (m, 1H), 2.50 (m, 1H), 2.35 (m, 1H), 2.31 (s, 3H), 2.27 (s, 3H), 2.20 (m, 2H), 2.03 (s, 3H); FABMS, m/z (rel intensity) 730(30), 495(2), 493(2), 481(2), 479(2), 463(4), 461(2), 449(4), 205(8), 204(8), 190(8); FABMS m/z 730.2493; B/E linked scan on m/z 729, m/z 711, 696, 683, 509, 495, 481, 479, 461, 449; optical relation $[\alpha]_D^{25}$ + 112° (c 0.01, CH_3OH);

Ecteinascidin 743 a beige solid, had: R_f = 0.58 (3:1 ethyl acetate-methanol), 0.44 (9:1 chloroform-methanol); HPLC retention time, 18.8 minutes (Whatman Partisil 10 ODS-3, 10 x 250 mm, 70:30 methanol-aqueous Tris (0.05 M), 2.8 mL/min); UV max (CH_3OH) 202 nm (ϵ81 000), 240 (sh) (15 000), 284 (6 600), 289 (6 400), (0.1 N HCl) 205 (76 000), 240 (sh) (12 000), 285 (7 500), 289 (7 200), (0.1 N KOH) 216 (50 000), 256 (12 700), 290 (9 000). IR max (CCl_4) 3549, 3530, 2992 (weak), 2929, 2848, 2803 (weak), 1764, 1739, 1597 (weak), 1511, 1501, 1460, 1445, 1425, 1365, 1350, 1195, 1160, 1115, 1102, 1098, 1082, 1058, 1048, 1024, 990, 950, 915, 907 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 6.62 (s, 1H), 6.48 (s, 1H), 6.46 (s, 1H), 6.03 (d, J = 1.2 Hz, 1H), 5.95 (d, J = 1.3 Hz, 1H), 5.71 (bs, exchanges, 1H), 5.14 (dd, J = 0.9, 11.3 Hz, 1H), 4.83 (bs, 1H), 4.50 (d, J = 3.3 Hz, 1H),

4.18 (d, J = 4.2 Hz, 1H), 4.06 (dd, J = 2.5, 11.3 Hz, 1H), 3.81 (s, 3H), 3.63 (s, 3H), 3.59 (bd, J = 4.4 Hz, 1H), 3.23 (bd, J = 6.5 Hz, 1H), 3.14 (ddd, J = 11, 10, 4 Hz, 1H), 2.91 (bd, J = 18 Hz, 1H), 2.88 (dd, J = 9, 18 Hz, 1H), 2.82 (m, 1H), 2.62 (ddd, J = 16, 10, 4 Hz, 1H), 2.49 (ddd, J = 16, 4, 4 Hz, 1H), 2.37 (bd, J = 13.9 Hz, 1H), 2.33 (s, 3H), 2.28 (s, 3H), 2.19 (s, 3H), 2.18 (d, J = 13.9 Hz, 1H), 2.04 (s, 3H); ^{13}C NMR (75.4 MHz and 125.7 MHz, $CDCl_3$) δ 9.6 (q), 15.7 (q), 20.4 (q), 24.0 (q), 28.7 (t), 39.6 (t), 41.3 (q), 42.1 (t), 42.1 (d), 54.8 (q), 55.0 (d), 55.9 (d), 57.7 (d), 57.8 (d), 60.2 (q), 61.3 (t), 64.6(s), 82.0 (d), 101.6 (t), 109.8 (d), 112.5, 114.1 (d), 115.9, 118.1(s), 120.9 (d), 121.9(s), 126.0(s), 129.2(s), 129.2(s), 131.5(s), 140.5(s), 141.3(s), 143.0(s), 144.3(s), 144.5(s), 145.1(s), 147.7(s), 168.3(s), 172.5(s); FABMS m/z (rel intensity) 744.2648 (100), 699.2766 (4), 613 (10), 495.2064 (15), 477.1978 (15), 475 (9), 463.1837 (25), 218(39), 204.1027 (71). LC/FABMS m/z (rel intensity) 744 (34), 495 (12), 493 (16), 477 (14), 475 (10), 463 (14), 234 (42), 218 (64), 204 (100), 189 (62), 174 (28), 160 (22); EIMS m/z 217.0737305, 191.0941620, 176.0696716. ESCA (mole percent) C(73.1), O(20.4), N(5.2), S(1.3), optical rotation $[\alpha]_D^{25}$ + 1140 (c 0.1, CH_3OH);

Ecteinascidin 745 had: R_f = 0.42 (3:1 ethyl acetate-methanol, 0.38 (9:1 chloroform-methanol). HPLC retention time, 29.8 min (Whatman Partisil 10 ODS-3, 10 x 250 mm, 70:30 methanol-aqueous Tris (0.05 M), 2.8 mL/min). UV max (CH_3OH) 202(ε52 000), 240(sh, 11 000), 281 (5 600), 287 (5 400), (0.1 N HCl), 204(51 000), 240 (sh, 9 500), 281 (5 200), 287 (5 200), (0.1 N KOH), 215 (36 000), 254 (8 500), 290 (5 900), 298 (5 800). IR (CCl_4 3554, 3535, 2955, 2927, 2871, 2855, 1770, 1744, 1518, 1507, 1270, 1238, 1195, 1163, 1088, 1056 cm^{-1}. ^1H NMR (360 MHz, $CDCl_3$) δ 6.62 (s, 1H), 6.52 (s, 1H), 6.47 (s, 1H), 6.02 (d, J = 1.2 Hz, 1H), 5.97 (d, J = 1,2 Hz, 1H), 5.74 (bs, exchanges, 1H), 5.14 (d, J = 11.2 Hz, 1H), 4.50 (bs, 1H), 4.29 (bt, J = 7 Hz, 1H), 4.22 (bs, 1H), 4.11 (dd, J = 11, 2 Hz, 1H), 3.80 (s, 3H), 3.68 (s, 1H), 3.63 (s, 3H), 3.31 (dd, J = 11, 2 1H), 3.23 (bs, 1H), 3.11 (m, 2H), 2.93 (m, 2H), 2.69 (m, 2H), 2.54 (m, 2H), 2.44 (d, J = 17 Hz, 1H), 2.33 (s, 3H), 2.28 (s, 3H), 2.18 (s, 3H), 2.13 (m, 1H), 2.04 (s, 3H); FABMS m/z (rel intensity) 746.2775 (100), 699 (8), 631 (8), 269 (8), 495 (19), 479 (42), 477 (52), 463 (36), 205 (64), 204 (64); LC/FABMS m/z (rel intensity) 746 (44), 495 (18), 477 (20), 463 (32),

218 (42), 204 (100), 189 (62), 176 (32), 160 (20), optical rotation $[\alpha]_D^{25}$ + 50° (c 0.1, CH_3OH); UV max (CH_3OH) 202(ε52 000), 240(sh, 11 000), 281 (5 600), 287 (5 400), (0.1 N HCl), 204(51 000), 240 (sh, 9 500), 281 (5 200), 287 (5 200), (0.1 N KOH), 215 (36 000), 254 (8 500), 290 (5 900), 298 (5 800);

Ecteinascidin 759A had: LC/FABMS m/z (rel intensity) 760 (26), 509 (12), 493 (12), 463 (24), 449 (16), 246 (26), 232 (32), 224 (62), 218 (52), 204 (100), 189 (56), 174 (18), 160 (16). R_f = 0.6 (3:1 ethyl acetate-methanol), 0.3 (9:1 chloroform-methanol); HPLC retention time, 11.0 min (Whatman Partisil 10 ODS-3, 10 x 250 mm, 70:30 methanol-aqueous tris (0.05 M), 2.8 mL.min); UV max (CH_3OH) 203 nm (ε x 43 000 43), 250 (sh) (6 500), 281 (3 000), 288 (2 600), (0.1 N HCl) 205 (44 000), 250 (sh) (7 600), 281 (4 500), 288 (4 400), (0.1 N KOH) 216 (39 000), 249 (9 300), 294 (4 600); IR (CCl_4) 3696, 3555, 3532, 2926, 2854, 1770, 1744, 1670, 1466, 1252, 1240, 1194, 1091 cm^{-1}; FABMS m/z (rel intensity) 760.2563 (58), 581 (25), 493 (16), 463 (80), 461 (100) optical rotation $[\alpha]_D^{25}$ + 130° (c 0.05, CH_3OH);

Ecteinascidin 759B had: LC/FABMS m/z (rel intensity) 760 (38), 508 (8), 493 (18), 463 (26), 475 (14), 248 (30), 234 (48), 218 (86), 204 (100), 189 (56), 176.(26), 160 (32). R_f = 0.6 (3:1 ethyl acetate-methanol), 0.3 (9:1 chloroform-methanol); HPLC retention time, 13.9 min (Whatman Partisil 10 ODS-3, 10 x 250 mm, 70:30 methanol-aqueous tris (0.05 M), 2.8 mL/min); UV max (CH_3OH) 208 nm (ε x 60), 288 (4 800), 293 (4 500), (0.1 N HCl) 209 (64 000), 288 (7 100), 293 (7 100), (0.1 N KOH) 220 (45 000), 260 (10 000), 298 (7 600); IR (CCl_4) 3555, 2933, 1770, 1743, 1590, 1514, 1465, 1453, 1446, 1431, 1368, 1356, 1330, 1288, 1264, 1240, 1193, 1163, 1124, 1110, 1089, 1032, 1006, 821 cm^{-1}; FABMS m/z (rel intensity) 760.2519 (100), 744 (71), 730 (19), 493 (29), 477 (43), 463 (76); optical rotation $[\alpha]_D^{25}$ + 167° (c 0.1, CH_3OH).

Example 3 Antibacterial Activity

In a disk diffusion assay against Micrococcus luteus, using 0.64 cm disks, the compounds of the present invention exhibited the following results:

Compound	Mass/disk (μg)	Zone of inhibition (mm)
Ecteinascidin 743	0.2	17
	0.1	14
	0.05	12

	0.02	9
	0.01	7
	0.005	trace
Ecteinascidin 745	40	7
	20	trace
Ecteinascidin 729	0.250	22
	0.125	20
	0.063	19

Bioactivity vs Micrococcus luteus. (0.64 cm disks).

Compound	Mass/disk μg)	Zone of inhibition (mm)
Ecteinascidin 759A	1	9
	0.1	-
Ecteinascidin 759B	1	15
	0.1	9
Ecteinascidin 770	1	14
	0.1	trace

Example 4 L1210 Tube Dilution Assay

The compounds of this invention were shown to inhibit the growth of L1210 mouse leukemia cells in vitro as shown in the following table. The L1210 tube dilution assay is described in detail in a publication by L. H. Li, et al., Cancer Research 39:4816 (1979). ID_{50} and ID_{90} refer to the concentration of ecteinascidin needed to inhibit cell growth by 50 and 90 percent, respectively.

Compound	ID_{50} (μg/mL)	ID_{90} (μg/mL)
Ecteinascidin 743	0.0005	0.0017
Ecteinascidin 745	0.088	0.19

Example 5 In Vivo Testing of Ecteinascidins Against P388 Leukemia

Compounds of this invention are also active in vivo against P388 leukemia in mice. The P388 mouse leukemia test is described in detail in a publication by G.L. Neil et al., Cancer Treatment Reports 63, 1971-1978 (1979). The results of three P388 mouse leukemia tests using different dosage schedules is shown below.

P388 in vivo

Compound	Dose (mg/kg)	T/C
Ecteinascidin 743	0.063	toxic
	0.031	161
Ecteinascidin 745	0.25	ca. 100

Example 6 (Isolation of Ecteinascidin 770)

E. turbinata (48 kg) is extracted in a procedure similar to Example 1. The methanol extract was partitioned into an upper "organic" layer and a lower "polar" layer by adding 1N aqueous $NaNO_3$ (4 L) and toluene (4 L, then 2 L). The toluene extracts were combined and evaporated under reduced pressure to produce a dark green oil (54 g). This material was triturated successively with hexane (10 x 50 mL, 10 x 20 mL), and dichloromethane (5 x 50 mL) to give a hexane triturate (50 g) and a dichloromethane triturate (2.1 g). A portion of the dichloromethane triturate (1.2 g) was purified by CCC (3 x 400 mg) using a cyclohexane-chloroform-methanol-water (1:2:4:2) solvent systen to give 4 fractions.

An early bioactive CCC fraction (500 mg) was chromatographed on a CHP-20 porous polymer MPLC column (18 x 350 mm) using a methanol-0.05 M tris (85.15) solvent system and UV detection (280 nm). Two bioactive peaks were collected. The first bioactive peak was purified by HPLC (70:30 methanol-0.05 M tris) to yield, after removal of buffer, ecteinascidin 759A (7 mg), ecteinascidin 759B (6 mg), and ecteinascidin 743 (5 mg). The second bioactive peak was purified by HPLC (70:30 methanol-0.05 M tris) to yield, after removal of buffer, ecteinascidin 770 (12 mg). R_f = 0.6 (3:1 ethyl acetate-methanol), 0.3 (9:1 chloroform-methanol); HPLC retention time, 12.0 (Whatman Partisil 10 ODS-3, 10 x 250 mm, 70:30 methanol-aqueous tris (0.05 M), 2.8 mL/min); FABMS, m/z 771.2682; $[\alpha]^{25}_D$ +52° (c 0.1, CH_3OH); UV max (CH_3OH) 342 nm (ε 3 200), 329 (3 900, 299 (22 000), 263 (25 000), 240 (58 000), 234 (55 000), 216 (66 000), (0.1 N HCl) 342 (4 900), 329 (5 700), 299 (24 000), 263 (29 000), 240 (58 000), 234 (57 000), 216 (71 000), (0.1 N KOH) 342 (3 700), 329 (4 900), 299 (22 000), 263 (28 000), 240 (58 000), 234 (57 000), 227 (57 000); IR (CCl_4) 3555, 3535, 3484, 2929, 2910, 1770, 1742, 1607, 1516, 1509, 1504, 1494, 1462, 1450, 1433, 1325, 1237, 1193 cm^{-1}; FABMS, *m/z* (rel intensity) 771 (48), 760 (8), 744 (20), 723 (12), 613 (14), 488 (12), 463 (14), 461 (20), 205 (50), 204 (80).

Anal. Calcd for $C_{40}H_{43}N_4O_{10}S$: 771.2700 (M + H).
Found: 771.2682.

Example 7 Chemical Degradations of Ecteinascidin 743

Part A. Treatment with KOH

Ecteinascidin 743 (1.0 mg, 1.3 μmol) is dissolved in methanolic

KOH (0.2 N) and stirred at room temperature. The reaction is followed by HPLC (Whatman Partisil 5 ODS-3, 4.6 X 250 mm, 70:30 : MeOH:0.05 M Tris, 1.5 mL/min. The product peak is observed immediately (3 min) by HPLC. After the reaction is complete (4.5 min), the reaction mixture is neutralized with methanolic HCl (0.5 M) and the solvent is removed under a stream of nitrogen. The product is taken up in methanol, filtered, and purified by HPLC to yield deacetylecteinascidin 743 (0.8 mg, 85%): Pale yellow solid; t_R = 7.0 min (Whatman Partisil 5 ODS-3, 4.6 x 250 mm, 70:30 : MeOH:0.05 M Tris, 1.5 mL/min); UV max (CH_3OH) 289 nm (ε 2 000), 283 (2 000), 203 (56 000), (0.1 N KOH) 300 (5 000), 295 (5 000), 255 (8 000) 215 (30 000); IR (CCl_4) 3536, 2951, 2926, 2878, 2855, 1743, 1270, 1239 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.63 (s,1) 6.49 (s,1), 6.41 (s,1), 5.94 (d,1,J = 1 Hz), 5.87, (d,1,J = 1 Hz), 5.09, (d,1,J = 10.7) 4.83 (s,1), 4.50 (m,1), 4.41 (s,1), 4.19 (d,1,J = 4.7 Hz) 4.01 (m,1), 3.82 (s,3), 3.65 (m,2), 3.59 (s,3), 3.23 (d,1,J = 6 Hz), 3.15 (m,1), 2.9 (1), 2.8 (1), 2.5 (1), 2.4 (1), 2.35 (s,3), 2.18 (s,3), 2.15 (s,3); FABMS, *m/z* (rel intensity) 702 (8), 218 (4), 204 (8); B/E linked scan FABMS *m/z* 702, *m/z* 702 → *m/z* (rel intensity) 701 (100), 688 (4), 672 (6), 654 (24), 453 (44), 451 (28), 433 (48) 421 (20).

Anal. Calcd for: $C_{37}H_{40}N_3O_9S$: 702.2485 (M + H).

Found: 702.2438 (HRFABMS).

Part B. Treatment with Lithium Aluminum Hydride

Ecteinascidin 743 (1.0 mg, 1.3 mol) is dissolved in cold diethyl ether (0.5 mL, 0 °C). Lithium aluminum hydride (ca. 0.5 mg, 13 μmol) is added, causing the immediate evolution of gas. The reaction mixture is stirred for 30 min, then warmed to 25 °C and stirred for 1 day. When HPLC analysis indicates no reaction after 1 day, another portion of LAH (ca. 0.5 mg) is added and gas is again produced. Addition of a third portion of LAH (ca. 0.5 mg) caused no further evolution of gas. HPLC analysis at this point is no longer practical due to excessive flocculant material in the reaction mixture. The mixture is stirred another 2 days, then quenched by slow addition of methanol (0.25 mL). Following removal of solvents under a stream of nitrogen, diethyl ether (10 mL) is added. The ethereal extract is filtered, washed with water (2 x 5 mL), and dried under a stream of nitrogen to give deacetylecteinascidin 743 (0.1 mg, 11%): pale yellow solid; t_R 7.2 min (Whatman Partisil 5 ODS-3, 4.6 x 250 mm,

70:30 : MeOH:0.05 M Tris, 1.5 mL/min); IR (CCl_4) 3536, 2951, 2926, 2874, 2855, 1740, 1270 cm^{-1}; FABMS, *m/z* (rel intensity) 744 (6), 702 (18), 453 (20), 451 (16), 435 (10), 433 (10), 421 (16), 419 (18), 218 (20), 204 (80).

Anal. Calcd for: $C_{37}H_{40}N_3O_9S$: 702.2485 (M + H).

Found: 702.2438 (HRFABMS).

Part C. Treatment with Ozone

Ecteinascidin 743 (2.3 mg, 3.1 μmol), in methanol (10 mL) is cooled to -78 °C. Ozone (Welsbach model T-816 ozonator) is bubbled through the solution until it turns light blue (3 min). Dimethyl sulfide (20 μL, 250 μmol), is added and the solution is stirred, slowly warmed (1 h) to room temperature, and stirred for 1 h. The product is dried under a stream of nitrogen, taken up in water (5 mL) and chloroform (5 mL), and the aqueous layer is extracted (3 x 5 mL) with chloroform to yield, after removal of solvent, an aqueous-soluble portion (3.1 mg) and an organic-soluble portion (<0.5 mg). HPLC analysis of the aqueous-soluble portion revealed the presence of a complex mixture. Attempts to isolate and purify the components of this mixture were unsuccessful.

Example 8 Derivatives of Exteinascidin 743

Part A. Preparation of Mono- and Di-O-methylecteinascidins 743

A freshly prepared ethereal solution of diazomethane [from Diazald (Aldrich), 0.25 mL] is added to a solution of ecteinascidin 743 (1 mg, 1.3 μmol) in methanol (0.25 mL). The reaction is followed by HPLC (Whatman Partisil 5 ODS-3, 4.6 x 250 mm, 70:30 : MeOH:0.05 M Tris 1.5 mL/min). An initial product (t_R = 11.2 min) is observed immediately (3 min). After 70 min a second product (t_R = 18.0 min) is observed in a ca. 1:1 ratio with the initial product. The reaction is stopped after 130 min by removing the solvents under a stream of nitrogen. Preparative HPLC (Whatman Partisil 10 ODS-3, 10 x 250 mm, 70:30 : MeOH:0.05 M Tris, 2.8 mL/min) of the reaction mixture afforded mono-O-methylecteinascidin 743 (0.3 mg, 29%) and di-O-methylecteinascidin 743 (0.5 mg, 48%).

Part B. Mono-O-methylecteinascidin 743

Beige solid; t_R = 11.2 min (Whatman Partisil 5 ODS-3, 4.6 x 250 mm, 70:30 : MeOH:0.05 M Tris, 1.5 mL/min; UV max (CH_3OH) 285 nm (ε 600), 201 (21 000), (0.1 N KOH) 287 (600); IR (CCl_4) 2859, 2855, 2851, 1770, 1741, 1520, 1264, 1226, 1196, 1126, 1089, 1053 cm^{-1}; ^1H

NMR ($CDCl_3$) δ 6.62 (s,1), 6.48 (s,1), 6.41 (s,1), 6.03 (s,1), 5.95 (s,1), 5.72 (s,1), 5.13 (d,1,J = 11.5 Hz), 4.81 (br s,1), 4.50 (m,2), 4.16 (d,1,J = 11.5 Hz), 4.81 (br s,1), 4.50 (m,2), 4.16 (d,1,J = 4.5 Hz), 4.05 (dd,1,J = 3,12 Hz), 3.80 (s,3) 3.76 (s,3), 3.60 (s,3), 3.20 (1), 2.88 (1), 2.85 (1), 2.63 (1), 2.50 (1), 2.33 (s,3), 2.27 (s,3), 2.18 (s,3), 2.03 (s,3); FABMS, *m/z* (rel intensity) 758 (10), 477 (22), 470 (28), 218 (12), 204 (12); B/E linked scan FABMS *m/z* 758 → *m/z* (rel intensity) 757 (100), 744 (10), 730 (14), 724 (14), 712 (20), 495 (88), 477 (20), 475 (18), 465 (12), 463 (28).

Anal. Calcd for: $C_{40}H_{44}N_3O_{10}S$ 758.2747 (M + H).

Found: 758.2754 (HRFABMS).

Part C. Di-O-methylecteinascidin 743

Beige solid, t_R = 18.0 min (Whatman Partisil 5 ODS-3, 4.6 x 250 mm, 70:30 : MeOH:).05 M Tris, 1.5 mL/min); UV max (CH_3OH) 280 nm (ϵ 500), 205 (10 000), (0.1 N KOH) 285 (700); IR (CCl_4) 2963, 2954, 2931, 2854, 1769, 1742, 1519, 1261, 1225, 1198, 1090, 1055, 1001 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 6.78 (s,1), 6.46 (s,1), 6.41 (s,1), 6.03 (s,1), 5.95 (s,1), 5.14 (d,1,J = 10.1 Hz), 4.82 (br s,1), 4.50 (m,1), 4.12 (dd,1,J = 2,5.5 Hz), 4.05 (dd,1,J = 2,11), 3.92 (s,3), 3.83 (s,3), 3.76 (s,3), 3.66 (m,1), 3.60 (m,3), 3.23 (m,1), 2.88 (m,2), 2.48 (m,1), 2.29 (s,3), 2.24 (s,3), 2.17 (s,3), 2.03 (s,3); FABMS, *m/z* (rel intensity) 772 (24), 509 (4), 491 (6), 477 (10), 218 (20), 204 (6); B/E linked scan FABMS *m/z* 772 → *m/z* (rel intensity) 771 (100), 758 (4), 742 (6), 726 (18), 509 (100), 491 (30), 489 (30), 479 (20), 477 (40).

Anal. Calcd. for: $C_{41}H_{46}N_3O_{10}S$: 772.2904 (M + H).

Found: 772.2891.

Part D. Preparation of Mono- and Dioxyecteinascidins 743

Aqueous hydrogen peroxide (30%, 0.5 μL) is added to a stirred methanolic solution (0.5 mL) of ecteinascidin 743 (1 mg, 1.4 μmol) cooled to 0°C. The reaction is followed by HPLC (Whatman Partisil 5 ODS-3, 4.6 x 250 mm, 70:30:MeOH:0.05 M Tris, 1.5 mL/min. The reaction mixture is stirred for 30 min, then warmed to 25 °C with stirring. After 1.5 h, an additional aliquot of hydrogen peroxide (30%, 5 μL) is added since no reaction has occurred. A third aliquot of hydrogen peroxide (30%, 5 μL) is added after 27 h and the reaction mixture is stirred for another 2 days. HPLC analyses revealed a complex mixture (ca. 16 peaks). Preparative HPLC purification of

this mixture affords monooxyecteinascidin 743 (0.2 mg, 20%) and dioxyecteinascidin 743 (0.4 mg, 38%).

Part E. Monooxyecteinascidin 743

Beige solid; t_R 7.0 min (Whatman Partisil 5 ODS-3, 4.6 x 250 mm, 70:30 : MeOH:0.05 M Tris, 1.5 mL/min); UV max (CH_3OH) 288 nm (ε 6 000), 281 (6 000), 235 (12 000), 201 (46 000), (0.1 N KOH) 291 (8 000), 260 (10 000), 213 (58 000); IR ($CHCl_3$) 1770, 1740 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.69 (s,1), 6.48 (s,1), 6.47 (s,1), 6.03 (s,1), 5.95 (s,1), 5.19 (s,1), 5.10 (d,1,*J* = 11.3 Hz), 4.80 (s,1), 4.73 (m,1), 4.54 (br s,2), 4.07 (d,1,*J* = 10 Hz), 3.82 (s,3), 3.63 (s,3), 3.03 (s,3), 2.86 (s,3), 2.26 (s,3), 2.03 (s,3); FABMS, *m/z* (rel intensity) 760 (16), 744 (10), 463 (6), 204 (22); B/E linked scan FABMS, *m/z* 760 → *m/z* (rel intensity) 744 (100), 743 (100), 713 (76), 698 (30), 465 (34).

Anal. Calcd for: $C_{39}H_{42}N_3O_{11}S$: 760.2540 (M + H).

Found: 760.2563 (HRFABMS).

Part F. Dioxyecteinascidin 743

Beige Solid; t_R 4.2 min (Whatman Partisil 5 ODS-3, 4.6 x 250 mm, 70:30 : MeOH:0.05 M Tris, 1.5 mL/min); UV max (CH_3OH) 282 nm (ε 12 000), 235 (22 000), 201 (78 000), (0.1 N KOH) 293 (14 000), 250 (20 000), 214 (63 000); IR ($CHCl_3$) 1770, 1740 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) methyl singlets: δ 3.89, 3.64, 2.33, 2.29, 2.10, 2.05; FABMS, *m/z* (rel intensity) 776 (4), 760 (4), 463 (42); B/E linked scan FABMS, *m/z* 766 → *m/z* (rel intensity) 759 (100), 758 (100), 713 (72), 463 (64), *m/z* 760 → *m/z* (rel intensity) 742 (100), 713 (34), 698 (28), 509 (36), 494 (45), 464 (30).

Anal. Calcd for: $C_{39}H_{42}N_3O_{12}S$: 776.2489)M + H).

Found: 776.2523 (HRFABMS).

Part G. Preparation of Monoacetylecteinascidin 743

Ecteinascidin 743 (2 mg, 2.8 μmol) is dissolved in dry dichloromethane (0.5 mL). Acetic anhydride (10 μL, 28 μmole) and pyridine (20 μL, 56 μmol) are added and the reaction is stirred at 25 °C. The reaction is followed by HPLC (Whatman Partisil 5 ODS-3, 4.6 x 250 mm, 70:30 : MeOH:0.05 M Tris, 1.5 mL/min. After 1 day, starting material is no longer detected. The solvents are removed under a stream of nitrogen and the product is purified by HPLC to yield acetylecteinascidin 743 (2.0 mg, 91%). White solid; t_R 11.5 min (Whatman Partisil 5 ODS-3, 4.6 x 250 mm, 70:30 : MeOH:0.05 M

Tris, 1.5 mL/min; UV max (CH_3OH) 282 nm (ε 7 000), 201 (84 000), (0.1 N KOH) 288 (11 000), 215 (62 000); IR (CCl_4) 3536, 2957, 2932, 2857, 1771, 1743, 1507, 1457, 1430, 1368, 1320, 1270, 1196, 1168, 1110, 1089, 1054, 1032, 915 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.61 (d,2), 6.45 (d,1), 6.01 (d,1), 5.95 (d,1), 5.71 (s,1), 5.12 (d,1), 4.81 (s,1), 4.49 (bs, 2), 4.18 (d,1), 4.04 (d,1), 3.61 (s,3), 3.54 (s,3), 3.21 (m,1), 3:12 (m,1), 2.88 (m,2), 2.61 (m,2), 2.49 (dd,1), 245 (d,1), 2.33 (s,6), 2.28 (s,3), 2.24 (s,3), 2.18 (s,6), 2.01 (d,3); FABMS, *m/z* (rel intensity) 786 (64), 744 (14), 495 (6), 493 (6), 477 (6), 463 (12), 461 (4), 218 (28), 204 (40); EIMS (70 eV), *m/z* (rel intensity) 768 (2), 458 (28), 443 (20), 430 (10), 416 (16), 403 (16), 385 (18), 375 (12), 360 (10), 274 (24), 259 (12), 245 (10), 231 (20), 217 (60), 203 (52), 189 (48), 174 (74), 160 (48), 146 (26), 133 (100), 115 (24); B/E linked scan FABMS, *m/z* 786 → *m/z* (rel intensity) 771 (12), 755 (10), 743 (14), 739 (18), 728 (4), 697 (4), 495 (50), 493 (30), 477 (20), 475 (18), 463 (56), 461 (18).

Anal. Calcd for: $C_{41}H_{44}N_3O_{11}S$: 766.2697 (M + H).

Found: 786.2712 (HRFABMS).

Part H. Preparation of Di- and Tetraacetylecetinascidins 743

Ecteinascidin 743 (0.5 mg, 0.7 μmol) is stirred in a solution of pyridine-acetic anhydride (2:1, 2 mL) for 2 days. The solvents are removed under a stream of nitrogen and the residue is taken up in water (5 mL) and chloroform (5 mL). The organic layer is washed with water (3 x 5 mL) and dried ($MgSO_4$), and the solvent is removed to yield a mixture of di- and tetraacetyl derivatives (0.5 mg, 85%): pale yellow solid; FABMS, *m/z* (rel intensity) 912 (10), 844 (8), 828 (30), 537 (2), 535 (2), 519 (4), 505 (8), 463 (4), 461 (4), 218 (18), 205 (42), 204 (36).

Part I. Preparation of *p*-Bromobenzoylecteinascidin 743

Ecteinascidin 743 (2 mg, 2.8 μmol), *p*-bromobenzoyl chloride (3.2 mg, 14 μmol, Aldrich), pyridine (2 μL, 25 μmol), and methylene chloride (0.5 ml) are combined and stirred at room temperature for 39 h. The reaction is followed by HPLC (Whatman Partisil 5 ODS-3, 4.6 x 250 mm, 70:30 : MeOH:0.05 M Tris, 1.5 mL/min). After 24 h additional amounts of *p*-bromobenzoyl chloride (ca. 3 mg) and pyridine (2 μL are added. The reaction is stopped after 39 h when there is no further change in the peak corresponding to the starting material. The product is purified by preparative HPLC (Whatman Partisil 10 ODS-3,

10 x 250 mm, 70:30 - 100:0 (step gradient) MeOH:0.05 M Tris, 2.8 mL/min) to yield *p*-bromobenzoylecteinascidin 743 (2.4 mg, 96%). White solid; UV max (CH_3OH) 280 nm (ε 8 000), 241 (31 000), 201 (90 000), (0.1 N KOH) 290 (8 000), 238 (31 000), 215 (50 000); IR (CCL_4) 3533, 2954, 2929, 2873, 2854, 1770, 1745, 1592, 1509, 1485, 1463, 1450, 1431, 1400, 1369, 1321, 1264, 1197, 1173, 1152, 1136, 1119, 1089, 1072, 1053, 1032 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 7.99 (d,2,*J* = 8.5 Hz), 7.61 (d,2,*J* = 8.5 Hz), 6.72 (s,1), 6.62 (s,1), 6.61 (s,1), 6.00 (s,1), 5.93 (s,1), 5.71 (br s,1), 5.14 (d,1,*J* = 11.2 Hz), 4.82 (s,1), 4.49 (br s,2), 4.17, (d,1,*J* = 4.7 Hz), 4.05 (dd,1,*J* = 11.2,2.2 Hz), 3.80 (s,3), 3.59 (d,1,*J* = 5.2 Hz), 3.53 (s,3), 3.22 (br d,1,*J* = 6), 3.15 (dd,1,*J* = 11, 5 Hz), 2.88 (br s,1), 2.85 (m,1), 2.82 (m,1), 2.65 (m,1), 2.52 (ddd,1,*J* = 16,4,4 Hz), 2.38 (br d,1,*J* = 14 Hz), 2.33 (s,3), 2.28 (s,3), 2.19 (m,1), 2.18 (s,3), 2.02 (s,3); FABMS *m/z* (rel intensity) 928 (6), 926 (5), 880 (1), 848 (1), 744 (1), 509 (2), 495 (6), 493 (4), 477 (10), 463 (22), 218 (30), 204 (52).

Anal. Calcd for: $C_{46}H_{45}{}^{81}BrN_3O_{11}S$: 928.1938 (M + H).

Found: 928.1954 (HRFABMS).

Example 9 Substructures of Ecteinascidin 743

From the spectral data on ecteinascidin 743, its derivatives and the analogous ecteinascidins one can assign substructures of the molecule. Additionally, the NMR data and especially the ^1H-^{13}C heteronuclear correlation NMR data allow correlation of the six CH_3 groups and the three aromatic CH groups. HRFABMS studies show fragmentation of ecteinascidin 743 into 4 pieces -- SCH_3, $C_{11}H_8NO_3$, $C_{15}H_{17}NO_5$, and $C_{12}H_{14}NO_2$. The molecular formulae of the three nitrogen-containing pieces, the very intense UV spectra, the aromatic ^{13}C NMR absorptions, and the aromatic ^1H NMR absorptions all suggest the presence of three trioxygenated tetrahydroisoquinoline units. The $C_{12}H_{14}NO_2$ fragment, the *m/z* 204 ion in the FAB mass spectrum, must contain the N-CH_3 that is absent in ecteinascidin 729, one aromatic hydroxyl, and the aromatic methyl (only two oxygens allowed in the formula).

Example 10 Bioactivities of the ecteinascidins

The antimicrobial activities (vs. *M. luteus*) and the cytotoxic activities (vs CV-1 cells) of the ecteinascidins and ecteinascidin 743 derivatives are shown in Tables 1 and 2. Within experimental error (the dilutions are accurate to within a factor of 2 error), the

activities of ecteinascidins 729 and 743 in this one assay are approximately equivalent and represent the most potent compounds in the series. Ecteinascidins 759A, 759B, and 770 are four to eight times less potent, and ecteinascidin 745 is some 32 times less potent than ecteinascidins 729 and 743. All of the derivatives of ecteinascidin 743 that are prepared have antimicrobial and cytotoxic activities. The monoacetyl derivatives has enhanced activity over the original compound, whereas the other derivatives are two to eight times less active.

The ecteinascidins are not antiviral for Herpes simplex virus type I (HSV-I) or vesicular stomatitis virus (VSV). Crude extracts have no antimicrobial activity against *Escherichia coli*, *Pencillium atrovenetum* and *Saccharomyces cerevisiae*. Ecteinascidin 743 is negative in the biochemical induction assay (BIA), an assay for DNA interaction.

The antimicrobial spectra of ecteinascidins 743 and 745 are shown in Table 3, and the antitumor activities of ecteinascidins 729 and 743 are compared in Table 4. The strong antitumor activity of the lower homologue ecteinascidin 729, is especially noteworthy. This compound is clearly responsible for much of the antitumor activity of the crude extract. It is intriguing to find ecteinascidin 745 (ring opened ecteinascidin 743) essentially devoid of activity (antimicrobial, cytotoxic, and antitumor).

TABLE 1

Anti-*M. luteus* Activities of the Ecteinascidins and Ecteinascidin 743 Derivatives (Zones of Inhibition in mm)

	mass (ng)/disk (6.35 mm)					
Compound	1600	800	400	200	100	50
Ecteinascidin 729	18	16	15	11	8	7
Ecteinascidin 743	23	20	14	13	12	9
Ecteinascidin 745	tr	-	-	-	-	-
Ecteinascidin 759A	10	8	tr	-	-	-
Ecteinascidin 759B	15	13	10	tr	-	-
Ecteinascidin 770	15	12	9	tr	-	-
Ecteinascidin 743 Derivatives						
Deacetyl	16	14	12	10	8	tr
Mono-O-methyl	14	14	11	8	-	-
Di-O-methyl	11	8	-	-	-	-
Monoacetyl	28	20	20	14	12	10
p-Bromobenzoyl	12	10	8	-	-	-

TABLE 2

Anti-CV-1 Activities of the Ecteinascidins and Ecteinascidin 743 Derivatives (Zones of Inhibition in mm)

	mass (ng)/disk (6.35 nm)					
Compound	1600	800	400	200	100	50
Ecteinascidin 729	18	16	13	10	10	9
Ecteinascidin 743	28	23	23	20	16	14
Ecteinascidin 745	14	9	-	-	-	-
Ecteinascidin 759A	16	16	10	tr	-	-
Ecteinascidin 759B	22	22	15	13	11	tr
Ecteinascidin 770	25	24	20	16	14	10
Ecteinascidin 743 Derivatives						
Deacetyl	16	15	11	-	-	-
Mono-O-methyl	26	25	23	20	22	13
Di-O-methyl	18	16	13	17	15	13
Monoacetyl	30	28	25	22	22	18
p-Bromobenzoyl	18	16	12	10	-	-

TABLE 3

Antimicrobial Spectrum of Ecteinascidins 743 and 745

(Zones of Inhibition in mm around 6.35 mm disk)

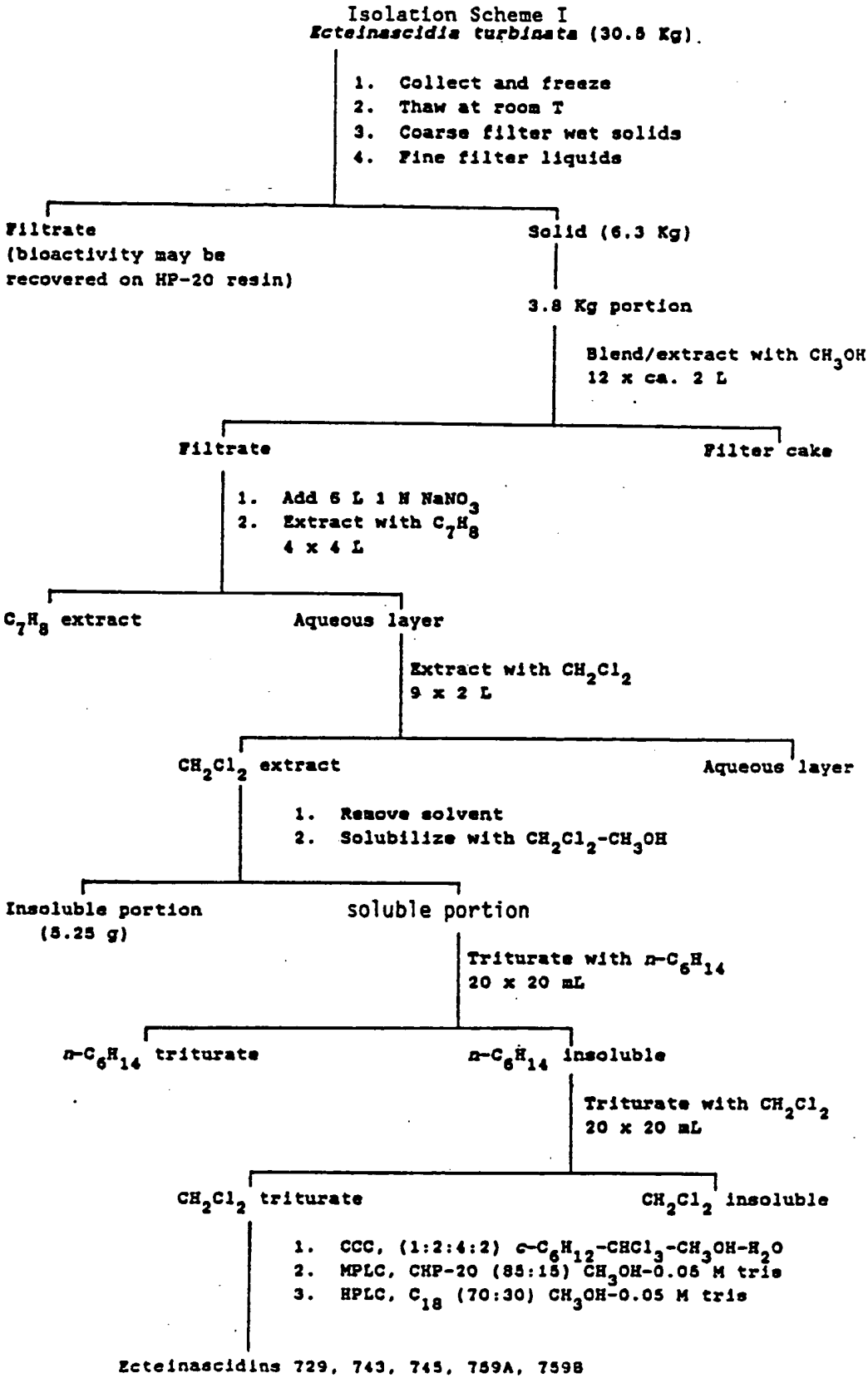
Microorganism	Ecteinascidin 743 (10 µg/disk	Ecteinascidin 745 (5 µg/disk
Bacillis subtilis	25	0
Bacillus subtilis syn	26	0
Klebsiella pneumoniae	20	0
Sarcina lutea	27	tr
Sarcina lutea sens.	35	18
Escherichia coli	11	0
Pseudomonas aeruginosa	0	0
Staphylococcus aureus	19	0
Mycobacterium avium	17	0
Streptococcus pyogenes	NT	tr
Saccharomyces cerevisiae	0	0
Penicillium oxalicum	10	0

TABLE 4

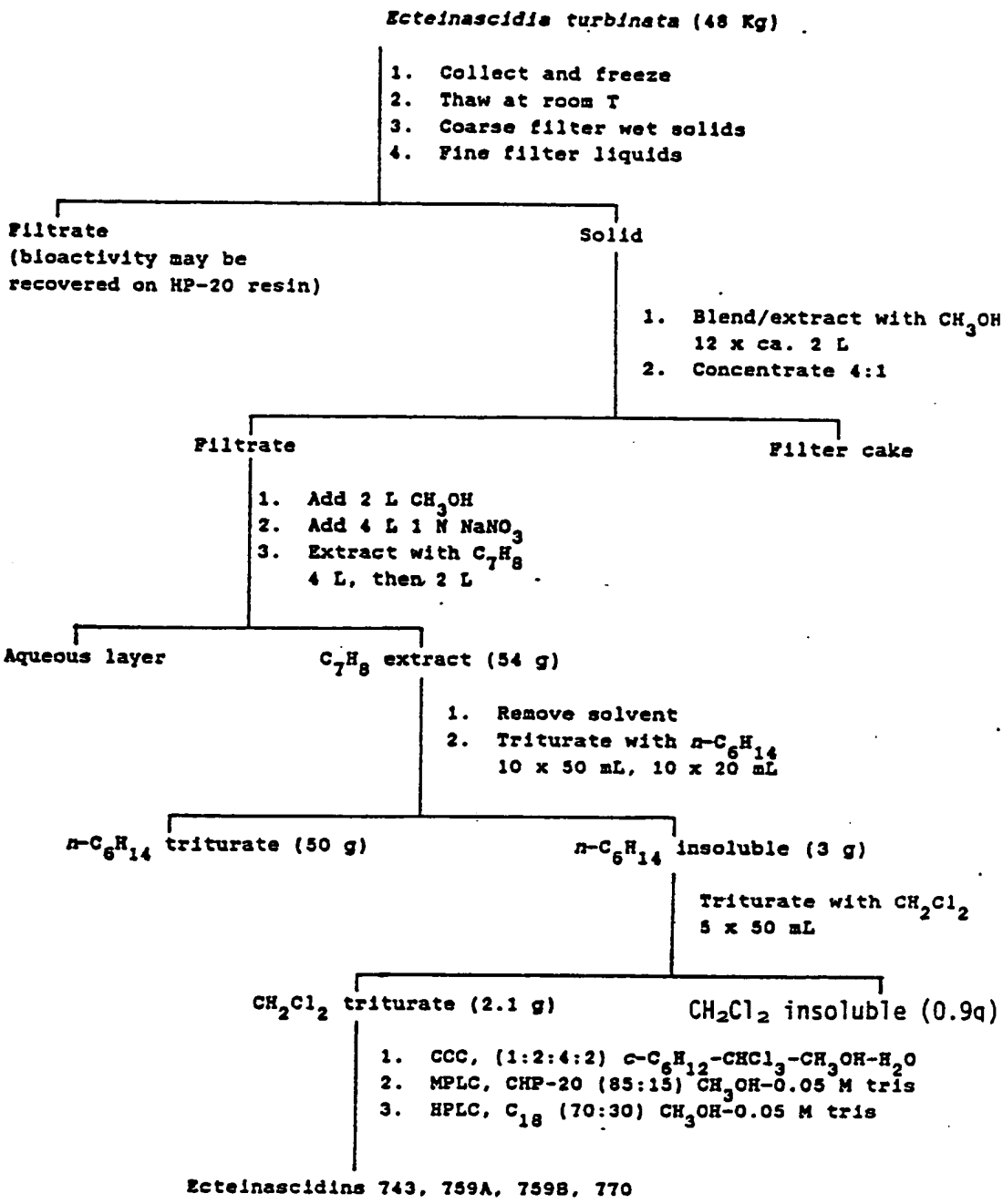
Antitumor Activity of Ecteinascidins 743 and 745 vs P388

Compound	T/C (%)	Dose (mg/kg)
Ecteinascidin 729	214	0.0038
Ecteinascidin 743	167	0.015
Ecteinascidin 745	111	0.25

CHART A1


Isolation Scheme I
Ecteinascidia turbinata (30.8 Kg)
1. Collect and freeze
2. Thaw at room T
3. Coarse filter wet solids
4. Fine filter liquids
Filtrate
(bioactivity may be
recovered on HP-20 resin)
Solid (6.3 Kg)
3.8 Kg portion
Blend/extract with CH3OH
12 x ca. 2 L
Filtrate
Filter cake
1. Add 6 L 1 M NaNO3
2. Extract with C7H8
4 x 4 L
C7H8 extract
Aqueous layer
Extract with CH2Cl2
9 x 2 L
CH2Cl2 extract
Aqueous layer
1. Remove solvent
2. Solubilize with CH2Cl2-CH3OH
Insoluble portion
(5.25 g)
soluble portion
Triturate with n-C6H14
20 x 20 mL
n-C6H14 triturate
n-C6H14 insoluble
Triturate with CH2Cl2
20 x 20 mL
CH2Cl2 triturate
CH2Cl2 insoluble
1. CCC, (1:2:4:2) c-C6H12-CHCl3-CH3OH-H2O
2. MPLC, CHP-20 (85:15) CH3OH-0.05 M tris
3. HPLC, C18 (70:30) CH3OH-0.05 M tris
Ecteinascidins 729, 743, 745, 759A, 759B

Isolation Scheme II



CLAIMS

1. Ecteinascidin 729, having the following characteristics: R_f = 0.28 (TLC, 5.02, 3:1 ethyl acetate-methanol), 0.26 (9:1 chloroform-methanol); HPLC retention time, 15.7 min (Whatman Partisil 10 ODS-3, 10 x 250 mm, 70:30 methanol-aqueous Tris (0.05 M), 2.8 mL/min); UV max (CH_3OH), 202 nm (ε 61 000), 244 (sh) (11 000), 283 (5 000), 289 (4 700), (0.1 N HCl) 204 (61 000), 244 (sh) (9 600), 283 (4 800), 289 (4 500), (0.1 N KOH) 215 (33 800), 258 (8 200), 290 (6 400); IR (CCl_4) 3555, 3535, 2953, 2927, 2855, 1770, 1742, 1504, 1466, 1462, 1454, 1432, 1369, 1239, 1196, 1168, 1122, 1100, 1086, 1054, 1032, 997, 960 cm^{-1}; ^1H NMR (360 MHz$CDCl_3$) δ 6.63 (s, 1H), 6.48 (s, 1H), 6.44 (s, 1H), 6.04 (d, J = 0.7 Hz, 1H), 5.95 (d, J = 0.9 Hz, 1H), 5.15 (d, J = 10.7 Hz, 1H), 4.84 (bs, 1H), 4.52 (bs, 1H), 4.48 (bs, 1H), 4.38 (d, J = 4.9 Hz, 1H), 4.04 (d, J = 11 Hz, 1H), 3.78 (s, 3H), 3.62 (s, 3H), 3.61 (m, 2H), 3.10 (m, 1H), 3.02 (bs, 1H), 2.90 (m, 1H), 2.80 (m, 1H), 2.60 (m, 1H), 2.50 (m, 1H), 2.35 (m, 1H), 2.31 (s, 3H), 2.27 (s, 3H), 2.20 (m, 2H), 2.03 (s, 3H); FABMS, m/z (rel intensity) 730(30), 495(2), 493(2), 481(2), 479(2), 463(4), 461(2), 449(4), 205(8), 204(8), 190(8); FABMS m/z 730.2493; B/E linked scan on m/z 729, m/z 711, 696, 683, 509, 495, 481, 479, 461, 449; optical relation $[\alpha]_D^{25}$ + 112° (c 0.01, CH_3OH)

2. Ecteinascidin 743, having the following characteristics: R_f = 0.58 (3:1 ethyl acetate-methanol), 0.44 (9:1 chloroform-methanol); HPLC retention time, 18.8 minutes (Whatman Partisil 10 ODS-3, 10 x 250 mm, 70:30 methanol-aqueous Tris (0.05 M), 2.8 mL/min); UV max (CH_3OH) 202 nm (ε81 000), 240 (sh) (15 000), 284 (6 600), 289 (6 400), (0.1 N HCl) 205 (76 000), 240 (sh) (12 000), 285 (7 500), 289 (7 200), (0.1 N KOH) 216 (50 000), 256 (12 700), 290 (9 000). IR max (CCl_4) 3549, 3530, 2992 (weak), 2929, 2848, 2803 (weak), 1764, 1739, 1597 (weak), 1511, 1501, 1460, 1445, 1425, 1365, 1350, 1195, 1160, 1115, 1102, 1098, 1082, 1058, 1048, 1024, 990, 950, 915, 907 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 6.62 (s, 1H), 6.48 (s, 1H), 6.46 (s, 1H), 6.03 (d, J = 1.2 Hz, 1H), 5.95 (d, J = 1.3 Hz, 1H), 5.71 (bs, exchanges, 1H), 5.14 (dd, J = 0.9, 11.3 Hz, 1H), 4.83 (bs, 1H), 4.50 (d, J = 3.3 Hz, 1H), 4.18 (d, J = 4.2 Hz, 1H), 4.06 (dd, J = 2.5, 11.3 Hz, 1H), 3.81 (s, 3H), 3.63 (s, 3H), 3.59 (bd, J = 4.4 Hz, 1H), 3.23 (bd, J = 6.5 Hz, 1H), 3.14 (ddd, J = 11, 10, 4 Hz, 1H), 2.91

(bd, J = 18 Hz, 1H), 2.88 (dd, J = 9, 18 Hz, 1H), 2.82 (m, 1H), 2.62 (ddd, J = 16, 10, 4 Hz, 1H), 2.49 (ddd, J = 16, 4, 4 Hz, 1H), 2.37 (bd, J = 13.9 Hz, 1H), 2.33 (s, 3H), 2.28 (s, 3H), 2.19 (s, 3H), 2.18 (d, J = 13.9 Hz, 1H), 2.04 (s, 3H); ^{13}C NMR (75.4 MHz and 125.7 MHz, $CDCl_3$) δ 9.6 (q), 15.7 (q), 20.4 (q), 24.0 (q), 28.7 (t), 39.6 (t), 41.3 (q), 42.1 (t), 42.1 (d), 54.8 (q), 55.0 (d), 55.9 (d), 57.7 (d), 57.8 (d), 60.2 (q), 61.3 (t), 64.6(s), 82.0 (d), 101.6 (t), 109.8 (d), 112.5, 114.1 (d), 115.9, 118.1(s), 120.9 (d), 121.9(s), 126.0(s), 129.2(s), 129.2(s), 131.5(s), 140.5(s), 141.3(s), 143.0(s), 144.3(s), 144.5(s), 145.1(s), 147.7(s), 168.3(s), 172.5(s); FABMS m/z (rel intensity) 744.2648 (100), 699.2766 (4), 613 (10), 495.2064 (15), 477.1978 (15), 475 (9), 463.1837 (25), 218(39), 204.1027 (71). LC/FABMS m/z (rel intensity) 744 (34), 495 (12), 493 (16), 477 (14), 475 (10), 463 (14), 234 (42), 218 (64), 204 (100), 189 (62), 174 (28), 160 (22); EIMS m/z 217.0737305, 191.0941620, 176.0696716. ESCA (mole percent) C(73.1), O(20.4), N(5.2), S(1.3), optical rotation $[\alpha]_D^{25}$ + 1140 (c 0.1, CH_3OH) or a derivative thereof.

3. Ecteinascidin 745, having the following characteristics: R_f = 0.42 (3:1 ethyl acetate-methanol, 0.38 (9:1 chloroform-methanol). HPLC retention time, 29.8 min (Whatman Partisil 10 ODS-3, 10 x 250 mm, 70:30 methanol-aqueous Tris (0.05 M), 2.8 mL/min). UV max (CH_3OH) 202(ε52 000), 240(sh, 11 000), 281 (5 600), 287 (5 400), (0.1 N HCl), 204(51 000), 240 (sh, 9 500), 281 (5 200), 287 (5 200), (0.1 N KOH), 215 (36 000), 254 (8 500), 290 (5 900), 298 (5 800). IR (CCl_4 3554, 3535, 2955, 2927, 2871, 2855, 1770, 1744, 1518, 1507, 1270, 1238, 1195, 1163, 1088, 1056 cm^{-1}. ^1H NMR (360 MHz, $CDCl_3$) δ 6.62 (s, 1H), 6.52 (s, 1H), 6.47 (s, 1H), 6.02 (d, J = 1.2 Hz, 1H), 5.97 (d, J = 1,2 Hz, 1H), 5.74 (bs, exchanges, 1H), 5.14 (d, J = 11.2 Hz, 1H), 4.50 (bs, 1H), 4.29 (bt, J = 7 Hz, 1H), 4.22 (bs, 1H), 4.11 (dd, J = 11, 2 Hz, 1H), 3.80 (s, 3H), 3.68 (s, 1H), 3.63 (s, 3H), 3.31 (dd, J = 11, 2 1H), 3.23 (bs, 1H), 3.11 (m, 2H), 2.93 (m, 2H), 2.69 (m, 2H), 2.54 (m, 2H), 2.44 (d, J = 17 Hz, 1H), 2.33 (s, 3H), 2.28 (s, 3H), 2.18 (s, 3H), 2.13 (m, 1H), 2.04 (s, 3H); FABMS m/z (rel intensity) 746.2775 (100), 699 (8), 631 (8), 269 (8), 495 (19), 479 (42), 477 (52), 463 (36), 205 (64), 204 (64); LC/FABMS m/z (rel intensity) 746 (44), 495 (18), 477 (20), 463 (32), 218 (42), 204 (100), 189 (62), 176 (32), 160 (20), optical rotation $[\alpha]_D^{25}$ + 50° (c

0.1, CH_3OH); UV max (CH_3OH) 202(ε52 000), 240(sh, 11 000), 281 (5 600), 287 (5 400), (0.1 N HCl), 204(51 000), 240 (sh, 9 500), 281 (5 200), 287 (5 200), (0.1 N KOH), 215 (36 000), 254 (8 500), 290 (5 900), 298 (5 800).

4. Ecteinascidin 759A, having the following characteristics: LC/FABMS m/z (rel intensity) 760 (26), 509 (12), 493 (12), 463 (24), 449 (16), 246 (26), 232 (32), 224 (62), 218 (52), 204 (100), 189 (56), 174 (18), 160 (16). R_f = 0.6 (3:1 ethyl acetate-methanol), 0.3 (9:1 chloroform-methanol); HPLC retention time, 11.0 min (Whatman Partisil 10 ODS-3, 10 x 250 mm, 70:30 methanol-aqueous tris (0.05 M), 2.8 mL.min); UV max (CH_3OH) 203 nm (ε x 43 000 43), 250 (sh) (6 500), 281 (3 000), 288 (2 600), (0.1 N HCl) 205 (44 000), 250 (sh) (7 600), 281 (4 500), 288 (4 400), (0.1 N KOH) 216 (39 000), 249 (9 300), 294 (4 600); IR (CCl_4) 3696, 3555, 3532, 2926, 2854, 1770, 1744, 1670, 1466, 1252, 1240, 1194, 1091 cm^{-1}; FABMS m/z (rel intensity) 760.2563 (58), 581 (25), 493 (16), 463 (80), 461 (100) optical rotation $[\alpha]_D^{25}$ + 130° (c 0.05, CH_3OH).

5. Ecteinascidin 759B, having the following characteristics: LC/FABMS m/z (rel intensity) 760 (38), 508 (8), 493 (18), 463 (26), 475 (14), 248 (30), 234 (48), 218 (86), 204 (100), 189 (56), 176 (26), 160 (32). R_f = 0.6 (3:1 ethyl acetate-methanol), 0.3 (9:1 chloroform-methanol); HPLC retention time, 13.9 min (Whatman Partisil 10 ODS-3, 10 x 250 mm, 70:30 methanol-aqueous tris (0.05 M), 2.8 mL/min); UV max (CH_3OH) 208 nm (ε x 60), 288 (4 800), 293 (4 500), (0.1 N HCl) 209 (64 000), 288 (7 100), 293 (7 100), (0.1 N KOH) 220 (45 000), 260 (10 000), 298 (7 600); IR (CCl_4) 3555, 2933, 1770, 1743, 1590, 1514, 1465, 1453, 1446, 1431, 1368, 1356, 1330, 1288, 1264, 1240, 1193, 1163, 1124, 1110, 1089, 1032, 1006, 821 cm^{-1}; FABMS m/z (rel intensity) 760.2519 (100), 744 (71), 730 (19), 493 (29), 477 (43), 463 (76); optical rotation $[\alpha]_D^{25}$ + 167° (c 0.1, CH_3OH).

6. Ecteinascidin 770, having the following characteristics: R_f = 0.6 (3:1 ethyl acetate-methanol), 0.3 (9:1 chloroform-methanol); HPLC retention time, 12.0 (Whatman Partisil 10 ODS-3, 10 x 250 mm, 70:30 methanol-aqueous tris (0.05 M), 2.8 mL/min); FABMS m/z 771.2682; $[\alpha]^{25}_D$ +52° (c 0.1, CH_3OH); UV max (CH_3OH) 342 nm (ε 3 200), 329 (3

900, 299 (22 000), 263 (25 000), 240 (58 000), 234 (55 ooo), 216 (66 000), (0.1 N HCl) 342 (4 900), 329 (5 700), 299 (24 000), 263 (29 000), 240 (58 000), 234 (57 000), 216 (71 000), (0.1 N KOH) 342 (3 700), 329 (4 900), 299 (22 000), 263 (28 000), 240 (58 000), 234 (57 000), 227 (57 000); IR (CCl_4) 3555, 3535, 3484, 2929, 2910, 1770, 1742, 1607, 1516, 1509, 1504, 1494, 1462, 1450, 1433, 1325, 1237, 1193 cm^{-1}; FABMS *m/z* (rel intensity) 771 (48), 760 (8), 744 (20), 723 (12), 613 (14), 488 (12), 463 (14), 461 (20), 205 (50), 204 (80).

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 4 :
C07G 11/00, A61K 35/00
// A61K 35/56

A2

(11) International Publication Number: WO 87/ 07610

(43) International Publication Date:
17 December 1987 (17.12.87)

(21) International Application Number: PCT/US87/01226

(22) International Filing Date: 1 June 1987 (01.06.87)

(31) Priority Application Numbers:
872,189
898,906
006,395

(32) Priority Dates:
9 June 1986 (09.06.86)
21 August 1986 (21.08.86)
23 January 1987 (23.01.87)

(33) Priority Country: US

(60) Parent Applications or Grants
(63) Related by Continuation
US 898,906 (CIP)
Filed on 21 August 1986 (21.08.86)
US 872,189 (CIP)
Filed on 9 June 1986 (09.06.86)
US 006,395 (CIP)
Filed on 23 January 1987 (23.01.87)

(71) Applicant *(for all designated States except US)*: UNIVERSITY OF ILLINOIS [US/US]; 506 South Wright Street, Urbana, IL 61801 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : RINEHART, Kenneth, L. [US/US]; 1306 South Carle Avenue, Urbana, IL 61801 (US). HOLT, Tom, G. [US/US]; 450 Paddock Drive West, Savoy, IL 61874 (US).

(74) Agent: WELCH, Lawrence, T.; Patent Law Department, The Upjohn Company, Kalamazoo, MI 49001 (US).

(81) Designated States: AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent), DK, FI, FR (European patent), GB (European patent), IT (European patent), JP, KR, LU (European patent), NL (European patent), NO, SE (European patent), US, US, US.

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: ECTEINASCIDINS 729, 743, 745, 759A, 759B AND 770

(57) Abstract

Novel compounds ecteinascidins 729, 743, 745, 759A, 759B, and 770 having antibacterial and antitumor properties.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**FR**	France	**ML**	Mali
AU	Australia	**GA**	Gabon	**MR**	Mauritania
BB	Barbados	**GB**	United Kingdom	**MW**	Malawi
BE	Belgium	**HU**	Hungary	**NL**	Netherlands
BG	Bulgaria	**IT**	Italy	**NO**	Norway
BJ	Benin	**JP**	Japan	**RO**	Romania
BR	Brazil	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CF	Central African Republic			**SE**	Sweden
CG	Congo	**KR**	Republic of Korea	**SN**	Senegal
CH	Switzerland	**LI**	Liechtenstein	**SU**	Soviet Union
CM	Cameroon	**LK**	Sri Lanka	**TD**	Chad
DE	Germany, Federal Republic of	**LU**	Luxembourg	**TG**	Togo
DK	Denmark	**MC**	Monaco	**US**	United States of America
FI	Finland	**MG**	Madagascar		

ECTEINASCIDINS 729, 743, 745, 759A, 759B AND 770

DESCRIPTION

The present application relates to novel compositions of matter. More particularly, the present application relates to novel antibacterial agents designated as ecteinascidin 729, 743, 745, 759A, 759B, and 770. These compounds are extracted from the marine tunicate Ecteinascidia turbinata, which is a well-known and readily available tropical marine invertebrate. Biological activity has been assigned previously to extracts of E. turbinata; see, for example, M. M. Sigal et al., "Anticellular and Antitumor Activity of Extracts from Tropical Marine and Vertebrates," in Food-Drugs from Sea Proceedings (1969), Youngken, H.W., Jr., Ed., Marine Technology Society,: Washington, D.C., 1970, pp 281-294; Lichter, W. et al., "Biological Activities Exerted by Extracts of Ecteinascidia turbinata," in Food-Drugs from the Sea Proceedings (1972), Worthen, L.R., Ed., Marine Technology Society: Washington, D.C., 1973, pp 117-127; Lichter, W., et al., "Inhibition of DNA Synthesis by Ecteinascidia turbinata Extracts (ETE)", in Food-Drugs from the Sea Proceedings, 1974, Webber, H.H., Ruggieri, G.D., Eds., Marine Technology Society: Washington, D.C., 1976, pp. 395-401; and Lichter, W. et al., "Immunomodulation by Extracts of Ecteinascidia turbinata", in Drugs and Food From the Sea, Kaul, P.N., Sindermann, C.J., Eds., The University of Oklahoma: Norman, OK, 1978, pp. 137-144.

INFORMATION DISCLOSURE

Extracts from Ecteinascidia turbinata are known, as described above. Certain of these extracts are known to have biological activity.

SUMMARY OF THE INVENTION

The present invention particularly provides:

(1) Ecteinascidin 729, having the following characteristics: R_f = 0.28 (TLC, 5.02, 3:1 ethyl acetate-methanol), 0.26 (9:1 chloroform-methanol); HPLC retention time, 15.7 min (Whatman Partisil 10 ODS-3, 10 x 250 mm, 70:30 methanol-aqueous Tris (0.05 M), 2.8 mL/min); UV max (CH_3OH), 202 nm (ϵ 61 000), 244 (sh) (11 000), 283 (5 000), 289 (4 700), (0.1 N HCl) 204 (61 000), 244 (sh) (9 600), 283 (4 800), 289 (4 500), (0.1 N KOH) 215 (33 800), 258 (8 200), 290 (6 400); IR (CCl_4) 3555, 3535, 2953, 2927, 2855, 1770, 1742, 1504, 1466, 1462, 1454, 1432, 1369, 1239, 1196, 1168, 1122, 1100, 1086, 1054, 1032, 997, 960 cm^{-1}; ^1H NMR (360 MHzCDCl$_3$) δ 6.63 (s, 1H), 6.48 (s,

1H), 6.44 (s, 1H), 6.04 (d, J = 0.7 Hz, 1H), 5.95 (d, J = 0.9 Hz, 1H), 5.15 (d, J = 10.7 Hz, 1H), 4.84 (bs, 1H), 4.52 (bs, 1H), 4.48 (bs, 1H), 4.38 (d, J = 4.9 Hz, 1H), 4.04 (d, J = 11 Hz, 1H), 3.78 (s, 3H), 3.62 (s, 3H), 3.61 (m, 2H), 3.10 (m, 1H), 3.02 (bs, 1H), 2.90 (m, 1H), 2.80 (m, 1H), 2.60 (m, 1H), 2.50 (m, 1H), 2.35 (m, 1H), 2.31 (s, 3H), 2.27 (s, 3H), 2.20 (m, 2H), 2.03 (s, 3H); FABMS, m/z (rel intensity) 730(30), 495(2), 493(2), 481(2), 479(2), 463(4), 461(2), 449(4), 205(8), 204(8), 190(8); FABMS m/z 730.2493; B/E linked scan on m/z 729, m/z 711, 696, 683, 509, 495, 481, 479, 461, 449; optical relation $[\alpha]_D^{25}$ + 112° (c 0.01, CH_3OH);

(2) Ecteinascidin 743, having the following characteristics: Beige solid, R_f = 0.58 (3:1 ethyl acetate-methanol), 0.44 (9:1 chloroform-methanol); HPLC retention time, 18.8 minutes (Whatman Partisil 10 ODS-3, 10 x 250 mm, 70:30 methanol-aqueous Tris (0.05 M), 2.8 mL/min); UV max (CH_3OH) 202 nm (ε81 000), 240 (sh) (15 000), 284 (6 600), 289 (6 400), (0.1 N HCl) 205 (76 000), 240 (sh) (12 000), 285 (7 500), 289 (7 200), (0.1 N KOH) 216 (50 000), 256 (12 700), 290 (9 000). IR max (CCl_4) 3549, 3530, 2992 (weak), 2929, 2848, 2803 (weak), 1764, 1739, 1597 (weak), 1511, 1501, 1460, 1445, 1425, 1365, 1350, 1195, 1160, 1115, 1102, 1098, 1082, 1058, 1048, 1024, 990, 950, 915, 907 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 6.62 (s, 1H), 6.48 (s, 1H), 6.46 (s, 1H), 6.03 (d, J = 1.2 Hz, 1H), 5.95 (d, J = 1.3 Hz, 1H), 5.71 (bs, exchanges, 1H), 5.14 (dd, J = 0.9, 11.3 Hz, 1H), 4.83 (bs, 1H), 4.50 (d, J = 3.3 Hz, 1H), 4.18 (d, J = 4.2 Hz, 1H), 4.06 (dd, J = 2.5, 11.3 Hz, 1H), 3.81 (s, 3H), 3.63 (s, 3H), 3.59 (bd, J = 4.4 Hz, 1H), 3.23 (bd, J = 6.5 Hz, 1H), 3.14 (ddd, J = 11, 10, 4 Hz, 1H), 2.91 (bd, J = 18 Hz, 1H), 2.88 (dd, J = 9, 18 Hz, 1H), 2.82 (m, 1H), 2.62 (ddd, J = 16, 10, 4 Hz, 1H), 2.49 (ddd, J = 16, 4, 4 Hz, 1H), 2.37 (bd, J = 13.9 Hz, 1H), 2.33 (s, 3H), 2.28 (s, 3H), 2.19 (s, 3H), 2.18 (d, J = 13.9 Hz, 1H), 2.04 (s, 3H); ^{13}C NMR (75.4 MHz and 125.7 MHz, $CDCl_3$) δ 9.6 (q), 15.7 (q), 20.4 (q), 24.0 (q), 28.7 (t), 39.6 (t), 41.3 (q), 42.1 (t), 42.1 (d), 54.8 (q), 55.0 (d), 55.9 (d), 57.7 (d), 57.8 (d), 60.2 (q), 61.3 (t), 64.6(s), 82.0 (d), 101.6 (t), 109.8 (d), 112.5, 114.1 (d), 115.9, 118.1(s), 120.9 (d), 121.9(s), 126.0(s), 129.2(s), 129.2(s), 131.5(s), 140.5(s), 141.3(s), 143.0(s), 144.3(s), 144.5(s), 145.1(s), 147.7(s), 168.3(s), 172.5(s); FABMS m/z (rel intensity) 744.2648 (100), 699.2766 (4), 613 (10), 495.2064 (15), 477.1978 (15), 475 (9), 463.1837 (25), 218(39), 204.1027 (71).